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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ^{4} **:**
A61K 39/395

A3

(11) International Publication Number: **WO 88/07869**

(43) International Publication Date: 20 October 1988 (20.10.88)

(21) International Application Number: PCT/BE88/00011

(22) International Filing Date: 18 April 1988 (18.04.88)

(31) Priority Application Number: 8700927

(32) Priority Date: 16 April 1987 (16.04.87)

(33) Priority Country: NL

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(81) Designated States: AT (European patent), BE (European patent), BJ (OAPI patent), CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), FR (European patent), GA (OAPI patent), GB (European patent), IT (European patent), JP, LU (European patent), ML (OAPI patent), MR (OAPI patent), NL (European patent), SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.
P *Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*
In English translation (filed in Dutch).

(88) Date of publication of the international search report:
17 November 1988 (17.11.88)

(54) Title: ANTI-INTERFERON-γ-ANTIBODIES AND THEIR THERAPEUTIC APPLICATION

(57) Abstract

Immunoglobulin with neutralizing antibody activity against interferon-γ of man or of an animal species for use as a therapeuthic agent, as an active substance for inhibiting inflammatory reactions, or preventing or inhibiting cerebral malaria, and medical or veterinary preparation comprising immunoglobulin acceptable excipient.

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"Anti-interferon-γ-antibodies and their therapeutic application"

Inflammatory responses are a component of many diseases of vertebrates including man. In its broadest meaning the term "inflammation" denotes local as well generalized responses. Local inflammation is characterized by vasodilatation, fluid transsudation from the vessels, infiltration of the tissues by leukocytes and, in some severe cases, intravascular thrombosis, damage to the blood vessels and extravasation of blood. The generalized inflammatory response, also denoted as acute phase response, is characterized by various reactions including increased body temperature, changes in the levels of various blood components (cellular as well as dissolved elements) and changes in blood pressure. In severe cases shock and death may occur.

Local and/or general inflammatory response occur as part of many diseases with endogenous or exogenous etiologies, in particular in (1) infections (local or generalized) with bacteria, viruses, fungi, parasites; (2) allergic reactions to common substances from the environment, drugs, erroneous blood transfusions; (3) as part of auto-immune diseases where the body's defense mechanism erroneously attacks its own tissues (e.g. rheumatoid arthritis, lupus erythematosus, demyelinating diseases, etc.); (4) rejection of tissue transplants.

In many cases local inflammation is self-limiting. However, in some instances local inflammation may go on for long periods of time, sometimes lifelong as in the case e.g. in rheumatoid arthritis. Likewise, general inflammation is usually self-limiting as in the case of common acute viral infections. However, in particular cases the response may be so severe that vascular shock and death occur. This is the case for example in the Waterhouse-Friderichsen syndrome, a complication of generalized infection with meningococci. Another example of a severe inflammatory response is the "Toxic Shock Syndrome" or "tampon disease" which occurs as a result of staphylococcal infection in females who use intravaginal tampons for menstrual hygiene.

The exact mechanisms underlying the various forms of inflammation are only partially known. Various endogenous mediators are involved which trigger or regulate the changes seen in the tissues or in the physiological parameters of patients suffering from the inflammatory diseases. These mediators belong to several categories: (1) small molecules such as histamine and prostaglandins; (2) serum proteins such as the complement factors; (3) certain hormones such as the corticoids; and (4) cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF) and interferons.

The present invention is constituted by the observation that the administration to vertebrate experimental animals of antibodies which bind and neutralize interferon-γ can block local as well as generalized inflammatory responses, particularly in lethal cerebral malaria.

The antibodies are proteins belonging to the class of immunoglobulins. They must be prepared such that they possess binding and neutralizing potential for the interferon-γ molecules of the specific animal species in which the inflammation is to be suppressed. In the examples described the experimental animal used was the mouse, and hence the antibodies were directed against mouse interferon-γ. Various techniques exist to prepare the suitable antibodies, in particular:

(1) as polyclonal antibodies, by isolation from the serum of animals which are immunized with the desired interferon-γ

(2) as monoclonal antibodies, by isolation from the supernatant of appropriate cell cultures known as hybridomas

(3) as monoclonal antibodies by isolation from serum or ascites fluid of animals inoculated with said hybridoma cells

(4) as monoclonal antibodies obtained by recombinant DNA-technology.

In order for the antibodies to be suitable as anti-inflammatory agents in vivo, they must have a chemical composition (or: an epitope recognition pattern) such that they not only bind to interferon-gamma, but also that they neutralize its biological effects on cells cultured in vitro, such as its antiviral effect and, in particular, its effects on macrophages. Incubation of cultured fibroblasts with homologous interferon-gamma renders them resistant against infection with viruses. Incubation of cultured macrophages with interferon-gamma renders them more reactive to chemoluminescence induction by a suitable inducer substance, such as bacterial lipopolysaccharide. Characterization of an anti-interferon-gamma antibody as an anti-inflammatory agent depends on its neutralizing ability on these in vitro biological effects. Addition of antibodies should remove the antiviral activity of interferon-gamma, but should also anihilate other biological activities.

Monoclonal or polyclonal antibodies can be administered by general route. The preparations are supplied in for instance a lyophilized and cooled form and may comprise an excipient, a preservative, an anti-oxidant, etc. Thus, in mice the preferential route of administration is by intraperitoneal injection. In other animal species, e.g. man, other routes of administration can be more suitable, e.g. intramuscular or intravenous injection. For local application on body surfaces which are permeable to proteins the preparation of anti-mouse interferon-gamma antibodies may take the form of gels, creams, ointments with the antibody as one of the active components.

Vertebrate animals that have been injected with monoclonal antibodies against homologous interferon-gamma show a decreased responsiveness to various exogenous inflammatory stimuli. The degree and duration of this inhibition depends on the dose and epitope-specificity of the antibody used. For long duration of the inhibitory effect it is essential that the injected antibody is eliminated slowly. Such elimination depends on the degree of foreignness between the administered antibody and the animals own immunoglobulins. Ideally, the antibodies should be of homologous origin.

Although the inflammatory responses are a normal part of the body's defenses against noxious agents, exagerated or protracted inflammation is often undesirable. In such cases, administration of antibodies against interferon-gamma will be useful in that it will relieve swelling, pain, bleeding, scar formation and other undesirable effects of inflammation.

Examples II-VI illustrate the effect of anti-interferon-gamma on local (examples II, III, IV, V) and generalized (example VI) inflammatory reactions elicited by bacterial lipopolysaccharides (LPS). LPS is a universal component of the cell wall of all Gram-negative bacteria. Although the polysaccharide part of the molecule differs from one bacterium to another, the lipoid core of the molecule has an invariant structure. In infections of vertebrate animals with Gram-negative bacteria, local as well as generalized inflammatory responses are largely due to the multiple biological effects of the lipoid core.

The usual local reactions are constituted by oedema and cellular infiltration. The usual generalized reaction is constituted by fever and the appearance of so-called acute phase proteins in the serum. In their extreme form the reactions to LPS are known as Shwartzman reactions, which may be local and/or generalized. They are characterized by intravascular trhombosis. The generalized Shwartzman reaction is often lethal.

Although the milder forms of inflammation caused by LPS are common features of naturally occurring infections with Gram-negative bacteria, both in animals and in man, the more severe (Shwartzman-like) forms are rather rare. The classical example is the Waterhouse-Friderichsen syndrome which occurs as a complication of infections with meningococci. It is characterized by generalized intravascular coagulation. Death is due to hypotension which results from bleeding in the adrenal glands. Maternal infections with Gram-negative bacteria are often accompanied by premature delivery and foetal death. It is assumed that a local Shwartzman type reaction, occurring in the placenta, is at the basis of this condition. Shwartzman-like lesions have also been noted in the kidneys of infants dead of E.coli enteritidis.

A Shwartzman-like reaction also occurs in infections of vertebrates with parasites. Thus patients suffering from malaria, an infection with Plasmodium falciparum, sometimes develop a lethal complication due to a thrombotic inflammatory lesion of the cerebral blood vessels. Example VII demonstrates that monoclonal antibodies against mouse interferon-gamma, when administered to mice, makes them resistant against this lethal complication of cerebral malaria.

Example VIII refers to an inflammatory response elicited as a result of a delayed type hypersensitivity (DTH) reaction. DTH -reactions occur as a result of repeated or sustained exposure of the body to certain proteins (antigens)which are foreign to the body. The pathogenesis is well-known: the foreign antigen stimulates multiplication and activity of T-DTH-cells which produce interferon-gamma. This interferon activates macrophages to become toxic for their environment. This resultsin inflammation. In the clinic DTH-reactions are extremely frequent. They occur under the form of inflammatory skin reactions (eczema) to a wide range of environmental agents. A dramatic form of DTH-reaction is constituted by the rejection of organ or tissue grafts in those cases where the donor is not histocompatible with the acceptor. Certain organs such as the kidney or the heart are relatively resistant to rejection. The skin, however, is extremely sensitive. Inhibition of skin graft rejections (as shown in Example VI) is considered as a severe test for a DTH-inhibitory drug.

Example I. Preparation of monoclonal antibodies against mouse interferon-γ

An 8-week old female Lou/Ca rat was immunized intraperitoneally with purified MuIFN-γ emulsified in incomplete Freund's adjuvant over a period of 37 weeks. A second set of intraperitoneal injections was given 17 1/2 weeks later. 21 1/2 weeks after the last injection, the rat was injected intravenously with 300,000 units of pure MuIFN-γ Four days after the last immunization, spleen cells were mixed at a 3:1 ratio with the non-secretor mouse myeloma cells Sp2/OAg14 and fused in the presence of 50% polyethylene glycol-4000 (Merck, Darmstadt, W-Germany) according to standard procedures. Fused cells were seeded in 96 microtiter plates (50 ul/well; Costar 3596, Cambridge) in Eagle's minimal essential medium (EMEM), supplemented with 10% fetal calf serum (FBS), 2 mM glutamine, 2.2 g/l of sodium bicarbonate and HT (hypoxanthine, 0.1 mM, thymidine, 16 μM). The following day wells received an additional equal volume of EMEM containing HAT-medium (aminopterine, 0,4 μM). Cultures showing hybridoma cell growth were screened for the presence of anti-MuIFN-γ antibodies using an enzyme-linked immunosorbent assay (ELISA) and for antiviral activity by the direct neutralization assay. Positive hybridomas were subcloned twice by limiting dilution in microtiter plates. Three stable hybridomas secreting IgG2a antibodies directed against Mu-IFN-γ were selected, designated F_1, F_2, and F_3.

Hybridoma cells (10^6) were injected into the peritoneal cavity of thymusless nude mice that had been retreated with 0.5 ml Pristan mineral oil (2,6,10,14-tetramethyl pentadecane, 96%). Ascitic fluid and serum was harvested 7 to 14 days after cell inoculation.

Ascitic fluid and sera of mice inoculated with hybridoma cells were tested for interferon-γ-binding activity using an enzyme-linked immunosorbent assay (ELISA). Microtiter plates (Nunc, Immuno plate I-96F, Denmar) were coated with 0.1 ug of pure mouse interferon- and incubated for at least 24 h at 4°C. After saturation of all unspecific binding sites with 0.5 % casein in phosphate-buffered saline, the plates were washed 3 times with 0.15 M NaCl, 0.05% Tween-20. Next, serial 0.5 $\log_{10}$ dilutions of ascitic fluids or serum containing antibodies to mouse interferon-γ were added (100 µl) and the plates were incubated for 2 h at room temperature. After washing, horse radish peroxidase-conjugated rabbit anti-rat immunoglobulin (Dako, Denmark) was added at a dilution of 1/1000 in phosphate-buffered saline (pH 7,4) containing 5% FBS, 5% mouse serum and 0.05% Tween-20. After incubation for 1 h at room temperature, the plates were washed and 100 ul of color reagent (0.4 mg/ml 0-phenylenediamine-2-HCl in 0.1 M phosphate /citrate buffer, pH 0.6 containing 0.02% H_2O_2 solution-30%) was added. The reaction was terminated after 50 min by addition of 50 µl of 4 M H_2SO_4. Optical absorption (OA) at 450 nm wavelength was taken as a measure of binding activity. Binding titers were expressed as the reciprocal of the dilution corresponding to 50% maximal OA-reading.

The neutralizing activity of the monoclonal antibodies was determined by incubating 0.5 $\log_{10}$ dilutions of the ascitic fluid or serum with 30 units/ml of murine interferon-g. The mixtures were incubated for 4 hours at 37 °C and then examined for their antiviral activity on mouse L929 cells, as well as for their macrophages activating effect in a macrophage chemoluminescence assay. These tests were performed using a macrophage cell line (LA5-9.8) which, on stimulation with lipopolysaccharide, produces reactive oxygen metabolites. This activity, termed the "respiratory burst", represents a mechanism whereby macrophages can cause inflammatory damage to tissues. The spontaneous degeneration of the labile oxygen components can be visualized as amplified light emission (chemoluminescence) by added luminescent substrates, e.g. lucigenin. If the macrophage cultures are first exposed to homologous interferon-g, light emission is greatly enhanced. This enhancement can be taken to measure macrophage activation by interferon-g. The results of the binding and neutralisation assays with the monoclonal antibodies are shown in Table I. This table shows that the preparations F_1, F_2, F_3 possess equivalent binding activity for interferon-g. However, F_2 and F_3 had a greater neutralizing activity for both the antiviral and the macrophage activating effect of interferon-g.

Table I. Neutralizing and binding capacity of monoclonal antibodies against murine interferon-g

Antibody tested	Antibody potency ($\log^{10}$ titer)		
Code/sample n°	Binding test	Neutralization test	
		Antiviral effect	Macrophage activation
F_1/2574 (serum)	6.0	3.25	2.0
F_2/2576 (serum)	4.9	5.5	4.6
2575 (ascites)	4.75	5.25	not tested
F_3/2578 (serum)	5.7	5.75	4.3
2577 (ascites)	5.7	5.75	not tested

Example II. Inhibition by monoclonal anti-interferon-γ-antibody of local inflammation

To demonstrate the inhibitory effect of anti-interferon-γ-antibody on local inflammation, a mouse footpad model was used.

Female NMRI mice, 6 weeks of age, were obtained from the Experimental Animal Centre of the University of Leuven, Lipopolysaccharide B (LPS) of *S.marcencens* was obtained from Difco (Detroit, Mich., U.S.A.).

Mice were injected into the right hind footpad with 5 ug of LPS (in 25 µl). Phosphate-buffered saline (PBS), pH 7.4, was injected as a control into the left hind footpad. Footpad swelling was measured daily with the aid of micrometer-calipers. Footpad swelling was calculated as % increase in footpad thickness, by comparing thickness of the LPS-injected footpad (L) with that of the contralateral injected footpad (C).

$$\% \text{ increase} = \frac{L-C}{C} \times 100.$$

A minimum of 4 mice was used for each experimental group. Percent inhibition of footpad swelling was calculated by the formula:

$$\text{Percent inhibtion} = \left(1 - \frac{\text{mean swelling in experimental group}}{\text{mean swelling in control group}}\right) \times 100$$

Each of the three monoclonal antibodies was tested for its effect on the course of local reaction in mice given a 5 µg dose of LPS in the footpad. The antibodies, either as serum of ascitic fluid were given to groups of 4 mice in a single intraperitoneal injection 24 h before the LPS challenge. The dose was adjusted so that equivalent amounts of interferon-γ-binding units were injected. Control mice received saline. The results are shown in Table II. It can be seen that antibody preparations F_2 and F_3 which possess neutralizing antibody, did not affect the footpad swelling reaction.

Table II. Effect of monoclonal antibodies against MuIFN-γ on LPS-induced footpad swelling reaction mice: comparison of neutralizing (F_2 and F_3) with poorly neutralizing (F_1) antibodies

Antibody injected[a]		Footpad swelling[b] (% ± S.E; N = 4)	
Code #	Neutraling titer (- $\log_{10}$)	Day 2	Day 3
F_1-serum	1.80	128 (9.79)	106 (15.28)
F_2-serum	5.40	14 (1.39)	11 (2.83)
F_2-ascitic fluid	5.25	12 (3.56)	7 (2.02)
F_3-serum	4.50	31 (5.09)	23 (6.99)
F_3-ascitic fluid	5.50	13 (1.09)	11 (2.09)
Control (9% saline)	-	135 (2.64)	121.5 (3.82)

[a] Antibodies were given i.p. 24 h before LPS challenge at a dose of 0.1 ml containing 4.55 $\log_{10}$ binding units/ml.

[b] Swelling measured on days 2 and 3 after LPS injection (5 µg).

Example III. Inhibition by monoclonal anti-interferon-γ antibody of local inflammation

For this Example the same technique for measuring local inflammation was used as in Example II. The design of this experiment was similar to that of the previous one, except that the antibodies were given on days 56, 42, 28, 21, 10 and 5 before local LPS challenge. Footpad swellings were recorded on days 2 and 3 post challenge. The results shown in Table II indicate that the inhibitory action of the monoclonal antibodies on the initial phase of the swelling reaction persisted for as long as 6 weeks.

Table III. Effects of monoclonal antibody to MuIFN-γ on LPS-induced footpad swelling reaction in mice: duration of effects

Time of antibody dose[a] (days before LPS)	%Inhibition (± S.E.) of footpad swelling on	
	day 2 post LPS	day 3 post LPS
98	-6 (± 25)	5 (± 22)
66	32 (± 17)	8 (± 23)
56	22 (± 30)	26 (± 27)
42	71 (± 10)	61 (± 20)
28	71 (± 10)	85 (± 2)
21	83 (± 2)	91 (± 2)
10	94 (± 3)	89 (± 2)
5	84 (± 3)	84 (± 2)

[a] Dose of antibody (F_3) : 0.1 ml i.p. (5,75 $\log_{10}$ neutralizing units/ml).

Each value represents the average % inhibition as calculated on 4 treated mice compared to 4 control mice.

Example IV. Inhibition of inflammation by monoclonal antibody to interferon-γ : dose-response relationship

For this experiment the same technique for measuring local inflammation was used as in Example II. The design of the experiment was also similar, except that the monoclonal antibody (F_3) was given in 3 different doses. Footpad responses were recorded on days 2 and 3. The results, as shown in Table IV, indicate that a minimal dose of antibody necessary to inhibit inflammation is $10^{3.75}$ neutralizing units per mouse. Since the mice weighed 25 g average, the minimal dose can also be estimated at $10^{5.35}$ neutralizing units per kg body weight.

Table IV. Effects of monoclonal antibody (F_3) to MuIFN-γ on LPS-induced footpad swelling reaction in mice: dose-response relationship

Antibody dose[a] $\log_{10}$ neutralizing units/mouse	% Inhibtion[b] of footpad swelling on day 2 post LPS	day 3 post LPS
4.75	94.0 (1.2)	91.0 (1.6)
3.75	63.0 (9.5)	58.0 (13.8)
2.75	5.5 (13.0)	-8.5 (29.4)

[a] Given in 0.1 ml intraperitoneal injections, 24 hr before LPS challenge.

[b] Each value represents the average % inhibition as calculated on 4 treated mice compared to 4 control mice. In parentheses: standard error.

Example V. Inhibition of inflammation by polyclonal antibodies against interferon-γ

Polyclonal antibodies were prepared as serum from rabbits immunized by serial injections of mouse interferon-γ . The antiserum was found to contain $10^{3.75}$ IFN-γ neutralizing units per ml. The ability of this serum to inhibit inflammation was tested with an experimental protocol that was similar to that described for Example II. The results, as shown in Table V, indicate that mice given 4 intraperitoneal injections of 0.1 ml of the antiserum (see timing in footnote to Table V) showed a significantly decreased footpad response to LPS.

Table V. Effect of polyclonal antibodies against mouse IFN-γ on LPS-induced footpad swelling reaction in mice

Treatment[a]	Footpad swelling[b] % ± S.E., N = 4	
	Day 2	Day 3
Polyclonal anti-IFN- antibody	57 (25.8)	55 (15.0)
Control (9% saline)	125 (6.8)	108 (2.8)

[a] Antibody or saline was given i.p. on days -1, 0 and 2 relative to LPS challenge, at a dose of 0.1 ml (=2.75 $\log_{10}$ neutralizing units per injection).

[b] Swelling measured on days 2 and 3 after LPS injection (5 µg).

Example VI. Prevention of lethal endotoxin shock by administration of anti-interferon-γ antibody

A generalized inflammatory reaction, known as the generalized Shwartzman reaction, or Sanarelli reaction was elicited in mice. The technique consists in giving to consecutive injections of the endotoxin of Gramnegative bacteria. The reaction is characterized by generalized coagulopathy apparent from petechiae on the ear skin of the mice. General shock is apparent from inactivity of the mice, and pilo-erection. In a high percent of the animals, the shock reaction is lethal.

The experiment illustrated by the data of Table V made use of the LPS of S.marcescens (see Example II). The mice received a local injection of 5 µg in the footpad, followed, after 24 hr, by an intravenous dose of 100 µg. From the results it can be seen that occurrence of disease was completely prevented by treatment with monoclonal antibody against interferon-γ .

Table VI. Prevention of lethal endotoxin shock[a] b administration of anti-interferon-γ antibody

	Control (no antibody)	Antibody-treated [b]
Total number	12	12
Number diseased[c]	12	0
Number dead	7	0

[a] Shock was provoked by a combination of 2 injections of LPS: a preparation dose of 5 µg in the footpad followed, after 24 hr by an intravenous provocative dose of 100 µg.

[b] Intraperitoneal injectioin of 0.1 ml (4.55 $\log_{10}$ binding units/ml) respectively 24 hrs before the local injection and simultaneously with the intravenous provocative dose.

[c] Inactivity, pilo-erection and petechiae.

Example VIa. Prevention of lethal celebral malaria in mice by monoclonal antibody against interferon-gamma

Cerebral malaria which develops in human patients infected with Plasmodium falciparum can experimentally be mimicked in mice (e.g. strain CBA/Ca mice), by inoculation with Plasmodium berghei. The disease appears in ca. 80% of infected mice, 1 to 2 weeks post inoculation and is characterized by convulsions, paralysis and ensueing death. The effectivenessof anti-interferon-gamma antibody in this model disease are illustrated by the results of Table VII.

Groups of mice (CBA/Ca - ANKA strain) were given intraperitoneal injections of monoclonal antibody F3 (see Example I) at times indicated in Table VII. The potency of the antibody was $10^{5.5}$ neutralizing units/ml and the dose was 0.1 ml per injection. The mice were infected by intraperitoneal inoculation of 10^5 plasmodium berghei-containing red blood cells. The results demonstrate that mice which had received a suitable schedule of injections of antibody F3 were protected against occurrence of lethal cerebral malaria.

Table VII. Protection of CBA/Ca mice against lethal cerebral malaria by administration of anti-interferon-gamma antibodies

Exp. N°	Treatment		Percent Incidence of lethal cerebral malaria
	Material injected	Time of injections⁻	
1	antibody F3	-6, 0, 7	0
	saline	-6, 0, 7	75
2	antibody F3	-7	29
		0	14
		4	14
		7	100
	saline		100

⁻ days relative to time of inoculation of Plasmodium berghei

Example VIII. Inhibition by anti-interferon-γ antibody of the rejection of skin transplants

It is known that skin allografts are generally rejected faster than organ allografts. Factors contributing to the rapid rejection of skin grafts are thought to include the extensive nonspecific inflammation caused by damage to the dermis and the large degree of ischemic necrosis in the immediate postgrafting period.

The ability of monoclonal antibody to MuIFN-γ to modify skin allograft survival was investigated using skin grafts of donor C57BL/6J mice applied into Balb/C recipients. Grafted mice received intraperitoneal injections of anti-MuIFN-γ or phosphate buffered saline pH 7.4 1 hour before grafting and daily thereafter for 10 days. Grafts were examined daily and rejection was scored when 100% of the area of the graft had undergone necrotic degeneration.

The results shown in Table VIII indicate that control Balb/C recipients of C57B1 skin allografts rejected their grafts starting on day 7. However, treatment with monoclonal antibody against MuIFN-γ for 10 days caused a delay in the onset of graft rejection.

Table VIII. The effect of monoclonal antibody to MuIFN- on survival of murine skin allografts[a]

Days after grafting	Surviving grafts			
	Antibody[b]-treated		Control (no antibody)	
	N/total	%	N/total	%
7	8/8	100	9/9	100
8	8/8	100	8/9	89
9	8/8	100	4/9	44
10	6/8	75	2/9	22
11	5/8	62.5	2/9	22
12	2/8	25	1/9	11
13	1/8	12.5	0/9	0
14	1/8	12.5	0/9	0
15	0/8	0	0/9	0

[a] Donor: C57B1/6J mice; recipients Balb/C mice.

[b] Intraperitoneal injection of 0.1 ml (5.0 log$_{10}$ neutralizing units/ml) 1 hour before grafting and daily for 10 days.

CLAIMS

1. Immunoglobulin with neutralizing antibody activity against interferon-γ of man or of an animal species for use as a therapeutic agent.

2. Immunoglobulin with neutralizing antibody activity against interferon-γ of man or of an animal species for use as an active substance for inhibiting inflammatory reactions.

3. Immunoglobulin with neutralizing antibody activity against interferon-γ of man of of an animal species resulting from infections, allergic reactions, auto-immuno reactions, transplant rejection reactions, DTH-reactions.

4. Immunoglobulin with neutralizing antibody activity against interferon-γ of man or of an animal species for preventing or inhibiting cerebral malaria.

5. Medical or veterinary preparation comprising an immunoglobulin as claimed in claims 1-3 and a pharmaceutical acceptable excipient.

6. Medical or veterinary preparation for inhibiting inflammatory reactions in man or animal species, comprising an immunoglobulin having neutralizing antibody activity against interferon-γ of man of said animal species, respectively, and a pharmaceutically acceptable excipient.

7. Medical or veterinary preparation for inhibiting or preventing cerebral malaria, comprising immunoglobulin with neutralizing antibody activity against interferon-γ of man, and a pharmaceutically acceptable excipient.

INTERNATIONAL SEARCH REPORT

International Application No PCT/BE 88/00011

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^4: A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^4	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Lymphokine Research, volume 6, no. 1, 1987, (New York, US), Symposium on the Molecular Basis of Lymphokine Action held at the Fifth International Lymphokine Workshop, 11-15 January 1987, (Clearwater Beach, Florida, US), A. Billiau et al.: "The role of interferons in local inflammation in mice as investigated by in vivo administration of recombinant interferons and monoclonal antibodies", see ref. no. 1432 --	1-7
X	EP, A, 0088540 (BIOGEN N.V.) 14 September 1983 see claim 32; page 55, line 33-37; page 38, line 32 - page 39, line 35 --	1-7
X	Science, volume 229, no. 4709, 12 July 1985, A.A.A.S., (Washington, D.C., US) S. Landolfo et al.: "Inhibition of interferon-gamma may suppress allograft reactivity by T lymphocytes in ./.	1-7

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search 9th September 1988	Date of Mailing of this International Search Report 24.10.88
International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	vitro and in vivo", see pages 176-179 --	
A	Chemical Abstracts, volume 87, no. 3, 18 July 1977, (Columbus, Ohio, US), Y. Riviere et al.: "Inhibition by anti-interferon serum of lymphocytic choriomeningitis virus disease in suckling mice", see page 390, abstract 19978t, & Proc. Natl. Acad. Sci. U.S.A. 1977, 74(5), 2135-9 --	1
P,X	The Journal of Immunology, volume 138, no. 12, 15 June 1987, The American Association of Immunologists, (US), H. Heremans et al.: "Regulation by interferons of the local inflammatory response to bacterial lipopolysaccharide", see pages 4175-4179 --------	1-7

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. BE 8800011

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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